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PATENT APPLICATION

~~IN THE UNITED STATES PATENT AND TRADEMARK OFFICE~~

| | |
|---|---|
| **In re Application of:** | **Jurg Nuesch, et al.** |
| **Application No.:** | New U.S. National Stage Application of PCT International Application No. PCT/EP00/07835 |
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| **Priority Date Claimed:** | 13 August 1999 (European Appl. No. 99 115 161.4) |
| **U.S. National Phase Filing Date:** | Date of mailing identified below |
| **Title:** | **PARVOVIRUS NS 1 VARIANTS** |



**EXPRESS MAIL CERTIFICATE**

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**SUBMISSION UNDER 35 U.S.C. §371 OF UNITED STATES PATENT APPLICATION (NATIONAL PHASE PROCEEDINGS) BASED ON INTERNATIONAL APPLICATION NO. PCT/EP00/07835 AND CLAIMING PRIORITY OF EUROPEAN PATENT APPLICATION NO. 99 115 161.4**

Commissioner for Patents
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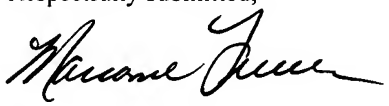
Sir:

Submitted herewith for filing under the provisions of 37 CFR 1.53 and 35 U.S.C. § 371 is the above-referenced patent application, based on International Patent Application No. PCT/EP00/07835 and

claiming priority of European Patent Application No. 99 115 161.4. A copy of the PCT International Application and related documents as originally filed are included. An English translation of the application as filed is also included. A translated International Preliminary Examination Report and International Search Report are included. Also included is a Preliminary Amendment, unsigned Declaration and Power of Attorney, a check in the amount of $430.00, and a transmittal letter.

Please direct correspondence relating to this application to Steven J. Hultquist, Intellectual Property Technology Law, P.O. Box 14329, Research Triangle Park, NC 27709, and direct telephonic communications relating to this application to Marianne Fuierer at (919) 419-9350.

Respectfully submitted,



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# Bescheinigung | Certificate | Attestation

EP 00/07835

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

JU

**Patentanmeldung Nr. | Patent application No. | Demande de brevet n°**

99115161.4

# PRIORITY DOCUMENT

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# Blatt 2 der Besch inigung
# Sheet 2 of the certificate
# ~~Page 2 de l'attestation~~

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# Parvovirus NS1 Variants

The present invention relates to parvovirus NS1 variants, DNAs coding for them and methods of producing the parvovirus NS1 variants. Furthermore, this invention concerns antibodies directed against the parvovirus NS1 variants as well as the use of the DNAs and the parvovirus NS1 variants.

Parvovirus designates a genus of the virus family Parvoviridae. The parvovirus genus comprises a number of small, icosaedric viruses that can replicate in the absence of a helper virus. Parvovirus contains a single-stranded DNA having a length of about 5.000 bp. At the 3' and 5' ends of the DNA there is one palindromic sequence each. The DNA codes for two capsid proteins, VP1 and VP2, as well as for two regulatory non-structure proteins, NS-1 and NS-2. The latter proteins are phosphorylated and show nuclear or both cytoplasmic and nuclear localization, respectively. NS1 is necessary for viral DNA replication and participates in the regulation of viral gene expression. Particularly, NS1 transactivates the promoter P38 and exhibits DNA-binding, helicase and DNA-nicking activities. Furthermore, NS1 induces cytotoxic and/or cytostatic stress in sensitive host cells.

Parvoviruses are usually well-tolerated by populations of their natural host, in which they persist without apparent pathological signs. This is due to both the protection of foetuses and neonates by maternal immunity, and the striking restriction of parvovirus replication to a narrow range of target proliferating tissues in adult animals. This host tolerance concerns especially rodent parvoviruses, for example the minute virus of mice (MVM) and H-1 virus in their respective natural hosts, namely mice and rats. In addition, humans can be infected with the latter viruses, without any evidence of

associated deleterious effects from existing epidemiological studies and clinical trials. On the other hand, it is known that certain parvoviruses, and especially rodent parvoviruses, are both oncotropic, i.e. accumulate preferentially in neoplastic versus normal tissues, and oncosuppressive, i.e. have a tumor-suppressive effect towards tumor cells, in various animal models. At least part of the oncosuppressive effect is thought to be due to a direct oncolytic action mediated by NS1. This oncosuppressive effect was also demonstrated against human tumor cells transplanted in recipient animals.

It is considered to use parvoviruses for therapeutic purposes. On the one hand, it seems to be of interest to use parvoviruses as vectors for therapeutic genes, i.e. for introducing such genes into the genome of cells. On the other hand, it is considered to use NS1 of parvoviruses as a toxin for treating tumoral diseases. However, initial experiments showed unsatisfactory results.

Therefore, it is the object of the present invention to provide a product by which parvoviruses and NS1 thereof, respectively, can be used for the above purposes.

According to the invention this is achieved by the subject matters defined in the claims.

The present invention is based on the applicant's findings that it is possible to interfere with the activities of parvovirus NS1 so as to shift the equilibrium existing between the DNA replication and transcription activities (a) and the cytotoxicity activity (b). In particular, he produced parvovirus NS1 variants in which the DNA replication and transcription activities (a) are reduced and eliminated, respectively, whereas the cytotoxicity activity (b) is maintained or raised. Moreover, he produced parvovirus NS1 variants in which the cytotoxicity activity (b) is reduced and eliminated, respectively, whereas the DNA replication and transcription activities (a) are

maintained or raised. Examples of such parvovirus NS1 variants are indicated in Table 1 and figure 1. In addition, the applicant recognized that the above parvovirus NS1 variants and expression vectors coding for them, particularly parvoviruses, respectively, are suitable for therapeutic purposes.

According to the invention, the applicant's findings are used to provide a parvovirus NS1 variant in which the equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b) is shifted.

The expression "parvovirus" comprises any parvovirus, particularly a rodent parvovirus, such as minute virus of mice (MVM) and H-1 virus.

The expression "the equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b) is shifted" refers to the fact that in a parvovirus NS1 variant according to the invention such an equilibrium is shifted as compared to the parvovirus NS1 wild-type. In particular, the equilibrium can be shifted to the effect that the DNA replication and transcription activities (a) are reduced and eliminated, respectively, whereas the cytotoxicity activity (b) is maintained or raised. The cytotoxicity activity (b) can also be reduced and eliminated, respectively, whereas the DNA replication and transcription activities (a) are maintained or raised. Such an equilibrium can be determined by various methods. As regards the determination of the DNA replication activity, reference is made e.g. to methods described in Legendre and Rommelaere, 1992, J. Virol. 66, 5705; Cotmore et al., 1992, Virology 190, 365; Cotmore et al., 1993, J. Virol. 67, 1579, Cotmore and Tattersall, 1994, Embo. J. 13, 4145. As to the determination of the transcription activity reference is made to methods described e.g. in Rhode and Richards, 1987, J. Virol. 61, 2807. Regarding the determination of the cytotoxicity activity reference is made to the below examples.

According to the invention parvovirus NS1 variants are preferred in which the shift of equilibrium is achieved by mutation of one or several phosphorylation sites. Particularly preferred are parvovirus NS1 variants which have a mutation at one or several of the phosphorylation sites 283, 363, 394 and 463. Even more preferred are the parvovirus NS1 variants S283A, T363A, T394A and T463A, which are indicated in Table 1 and figure 1. In S283A, a serine is exchanged by an alanine at position 283, in T363A a threonine is exchanged by alanine at position 363, in T394A a threonine is exchanged by alanine at position 394 and in T 463A a threonine is exchanged by alanine at position 463.

A further subject matter of the present invention relates to a nucleic acid, particularly a DNA, which codes for an above parvovirus NS1 variant. Such a DNA comprises preferably:

(a) the DNA of fig. 1.1, 1.2, 1.3 and 1.4, respectively

(b) a DNA hybridizing with the DNA from (a), said DNA comprising the mutated phosphorylation site of the DNA from (a), or

(c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.

The DNA of (a) was deposited with DSMZ (Deutsche Sammlung von Mikroorganismen and Zellkulturen) on Aug. 11, 1999, i.e. fig. 1.1 as Escherichia coli pRSV-NS:S283A under DSM 12994, fig. 1.2 as Escherichia coli pRSV-NS:T363A under DSM 12995, fig. 1.3. as Escherichia coli pRSV-NS:T394A under DSM 12996 and fig. 1.4 as Escherichia coli pRSV-NS:T463A under DSM 12997.

The expression "hybridizing DNA" refers to a DNA which hybridizes with a DNA from (a) under normal conditions, particularly at 20(C below the melting point of the DNA. In this connection, the expression "hybridizing" refers to conventional hybridization conditions, preferably to hybridization conditions where 5xSSPE, 1 % SDS, 1xDenhardt's solution are used as solution and the hybridization temperatures are between 35(C and

70(C, preferably 65(C. The hybridization is followed by a wash step first carried out with 2xSSC, 1 % SDS and then with 0.2xSSC at temperatures between 35(C and 70(C, preferably at 65(C. Furthermore, reference is made to Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd edition, Cold Spring Harbor Laboratory Press, cold Spring Harbor NY (1989).

A DNA according to the invention can be present in a vector and expression vector, respectively. A person skilled in the art is familiar with examples thereof. In the case of an expression vector for E. coli these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b, T7 based expression vectors and pQE-8. For the expression in yeast, e.g. pY100 and Ycpad1 have to be mentioned while e.g. pKCR, pEFBOS, cDM8, pMSCND, and pCEV4 have to be indicated for the expression in animal cells. The baculovirus expression vector pAcSGHisNT-A is especially suitable for the expression in insect cells.

In a preferred embodiment, the vector containing the DNA according to the invention is a virus, e.g. an adenovirus, vaccinia virus, an AAV virus or a parvovirus, such as MVM or H-1, a parvovirus being preferred. The vector may also be a retrovirus, such as MoMULV, MoMuLV, HaMuSV, MuMTV, RSV or GaLV.

For constructing expression vectors which contain the DNA according to the invention, it is possible to use general methods known in the art. These methods include e.g. in vitro recombination techniques, synthetic methods and in vivo recombination methods as described in Sambrook et al., supra, for example.

Furthermore, the present invention relates to host cells which contain the above described vectors. These host cells include bacteria, yeast, insect and animal cells, preferably mammalian cells. The E. coli strains HB101, DH1, x1776, JM101, JM109, BL21, XL1Blue and SG 13009, the yeast strain Saccharomyces cerevisiae and the animal cells L, A9, 3T3, FM3A, CHO, COS,

Vero, HeLa and the insect cells sf9 are preferred. Methods of transforming these host cells, of phenotypically selecting transformants and of expressing the DNA according to the invention by using the above described vectors are known in the art.

Moreover, the present invention relates to antibodies which specifically recognize an above describe parvovirus NS1 variant, i.e. the region of the parvovirus NS1 variant where the mutation responsible for the shifted equilibrium, particularly a mutated phosphorylation site, is located. The antibodies can be monoclonal, polyclonal or synthetic antibodies or fragments thereof, e.g. Fab, Fv or scFV fragments. Preferably monoclonal antibodies are concerned. For the production it is favorable to immunize animals - particularly rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above parvovirus NS1 variant or with fragments thereof. Further boosters of the animals can be effected with the same parvovirus NS1 variant or with fragments thereof. The polyclonal antibody can then be obtained from the animal serum and egg yolk, respectively. The monoclonal antibody can be obtained according to standard methods, reference being made particularly to the method by K÷hler and Milstein (Nature 256 (1975), 495) and GalfrÚ (Meth. Enzymol. 73 (1981), 3). In this case, mouse myeloma cells are fused with spleen cells originating from the immunized animals. Antibodies according to the invention can be used in many ways, e.g. for the immunoprecipitation of the above described parvovirus NS1 variants or for the isolation thereof. The antibodies can be bound in immunoassays in liquid phase or to a solid carrier. In this connection, the antibodies can be labeled in various ways. The person skilled in the art is familiar with suitable markers and labeling methods. Examples of immunoassays are ELISA and RIA.

The present invention provides parvovirus NS1 variants in which the equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b) is shifted. In

particular, parvovirus NS1 variants are provided which have a reduced or no cytotoxicity activity, whereas the DNA replication and transcription activities are maintained or increased. Parvovirus NS1 variants are also provided in which the DNA replication and transcription activities are reduced and eliminated, respectively, whereas the cytotoxicity activity is maintained or raised. Thus, the present invention provides products which are suitable for therapeutic purposes. In particular, expression vectors according to the invention, e.g. parvoviruses, can be used for gene-therapeutic measures. Moreover, parvoviruses NS1 variants according to the invention are suitable as toxins, e.g. for treating tumoral diseases.

Therefore, a kit is also provided for the application of the present invention. This kit comprises the following:

(a) a parvovirus NS1 variant according to the invention,
(b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
(c) an antibody according to the invention, as well as
(d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls.

Of component (a) to (d) one or more representatives can be present each.

Brief description of the drawings

Fig. 1 shows the DNA and amino acid sequences of parvovirus NS1 variants according to the invention (fig. 1.1, 1.2, 1.3 and 1.4) as compared to a parvovirus NS1 wild-type. In this connection, the mutated sites in the parvovirus NS1 variants according to the invention are labeled each.

The present invention is explained by the examples.

**Example 1: Preparation and purification of NS1 variants according to the invention**

The DNA of the NS1 variant S283A according to the invention was provided as an EcoRV to BstEII fragment obtained by chimeric PCR using two mutagenic primers. This fragment was then inserted into the corresponding cleaved expression vector pTHisNS1 (Nuesch et al., Virology 209, (1995), 122) to obtain pTHis NS1:S283A. Such a vector codes for a fusion protein comprising 6 histidine residues (N terminus partner) and S283A of Fig. 1 (C terminus partner). For expression and purification of S283A the NS1 gene under control of the bacteriophage T7 promoter was transferred into vaccinia virus and expressed in eucaryotic cells by double infection together with vTF7-3 (a vaccinia virus expressing the bacteriophage T7 DNA polymerase). 18 hrs post infection cells were harvested and nuclear extracts prepared. The histidine tagged S283A was then purified by affinity chromatography on Ni-NTA agarose and analyzed by 10 % SDS-PAGE (Nuesch et al., supra).

It showed that a parvovirus NS1 variant according to the invention can be prepared in highly pure form.

The NS1 variants T363A, T394A, and T463A were also produced and purified in the same way.

**Example 2: Preparation and detection of an antibody according to the invention**

Tubes were coated with purified NS1 variants prepared as in example 1 and monoclonal antibodies (e.g. scFv) specifically binding to S283A were isolated from human synthetic VH+VL scFV phage library (Griffith et al., EMBO J., 13, (1994), 3245) according to standard panning protocols after >5 isolation and amplification procedures. The variable region of the isolated scFv harbored in the phagemid were sequenced to identify NS1

variant interacting partner proteins harboring such binding motifs from comparison with known genes in the gene bank.

It showed that monoclonal antibodies according to the invention can be isolated.

In addition, the NS1 variants were used for immunization of animals in order to obtain poly- or monoclonal antibodies.

**Example 3: Characterization of the parvovirus NS1 variants S283A, T363A, T394A and T463A according to the invention**

The characterization of the parvovirus NS1 variants comprised the determination of the DNA replication, transcription, cytotoxicity, DNA binding, nicking and helicase activities. Known methods were used for this purpose (cf. description, supra). As regards the determination of the helicase activity reference is made to Stahl et al. 1986, EMBO J. 5, 1999. As to the determination of the nicking activity reference is made to Christensen et al., 1997, J. Virol. 71, 1405 and Nuesch et al., 1995, supra. Regarding the determination of the DNA binding reference is made to Cotmore et al. 1995, J. Virol. 69, 1652. As far as the determination of the cytotoxicity activity is concerned, the following steps were carried out:
NS1 variants were transferred into an expression vector containing the NS1 gene under the control of the parvovirus MVMP4 promoter (genuine promoter driving the non-structural genes of MVM), and the green fluorescent protein (EGFP) under control of an additional promoter. These constructs were then transfected into A9 cells using lipofectamine (GibcoBRL) according to the manufacturer's instruction and the impact of the NS1 variant on the viability of the cells tested in time course experiments. Transfected cells were identified by fluorescence of the EGFP. Toxic effects were determined in comparison to wild type NS1 or a vector containing no NS1 gene

as a function of time as well as a measure of cytopathic changes on the cell morphology.

The data indicated in Table 1 were obtained:

**Table 1**

| | S283A | T363A | T394A | T463A | wt |
|---|---|---|---|---|---|
| P38-TA | + | - | - | ++++ | ++++ |
| ACCA | + | ++++ | ++ | ++ | ++ |
| Nick-1 | + | - | - | +++ | +++ |
| Nick-2 | +++ | - | - | ++++ | ++++ |
| Nick-3 | ++ | - | - | | ++++ |
| Heli | ++ | - | (+) | ++++ | ++++ |
| Rep | + | - | - | + | ++++ |
| Cyto | ++++++ | ++ | +++ | (+) | +++ |

**Example 4: NS1 variants' expression after transduction using recombinant viral vectors**

NS1 expression cassettes containing the NS1 variants according to the invention under control of the parvoviral P4 promoter and a 3'untranslated region from parvovirus MVM to ensure stability and translation of the gene product, were transferred either in a parvovirus genome background as exemplified in example 3, or a heterologous viral genome background, such as vaccinia virus (example 1) or adenovirus. Promoter and terminator regions were exchanged according to the requirements. The nucleic acids containing the NS1 variants were then packaged either in vivo (after transient transfection into eucaryotic cells) or in vitro and the packaged transducing particles were isolated. These transducing units containing NS1 variants were used either for studies concerning gene regulation in tissue culture or animals, but also as therapeutic agents either alone or in combination with other agents (such as cytokines) in gene and cancer therapy

approaches.

## Claims

1. A parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b).

2. The parvovirus NS1 variant according to claim 1, wherein the activities (a) are reduced and eliminated, respectively, and activity (b) is maintained or increased.

3. The parvovirus NS1 variant according to claim 1, wherein activity (b) is reduced and eliminated, respectively, and the activities (a) are maintained or increased.

4. The parvovirus NS1 variant according to any one of claims 1 to 3, wherein one or several phosphorylation sites are mutated.

5. The parvovirus NS1 variant according to claim 4, wherein the mutations are located at sites 283, 363, 394 and/or 463.

6. The parvovirus NS1 variant according to claim 4 or 5, namely the NS1 variants S283A, T363A, T394A, and T463A.

7. A DNA, coding for the parvovirus NS1 variant according to any one of claims 1 to 6.

8. The DNA according to claim 7, wherein the DNA comprises:

   (a) the DNA of figure 1,
   (b) a DNA hybridizing with the DNA from (a), said DNA comprising the mutated phosphorylation site of the DNA from (a), or
   (c) a DNA related to the DNA from (a) or (b) via the degenerated genetic code.

9. An expression vector, comprising the DNA according to claim 7 or 8.

10. A transformant, containing the expression vector according to claim 9.

11. A method of producing the parvovirus NS1 variant according to any one of claims 1 to 6, comprising the culturing of the transformant according to claim 10 under suitable conditions.

12. An antibody, directed against the parvovirus NS1 variant according to any one of claims 1 to 6.

13. Kit comprising:
(a) a parvovirus NS1 variant according to the invention,
(b) a DNA according to the invention, e.g. an expression vector, particularly a parvovirus,
(c) an antibody according to the invention, as well as
(d) conventional auxiliary agents, such as solvents, buffers, carriers, markers and controls,

wherein of components (a) to (d) one or more representatives can be present each.

14. Use of the parvovirus NS1 variant according to any one of claims 1 to 6 as a toxin for treating tumoral diseases.

15. Use of the DNA according to claim 9 as a vector for gene therapy.

Fig. 1

Wild-type NS1

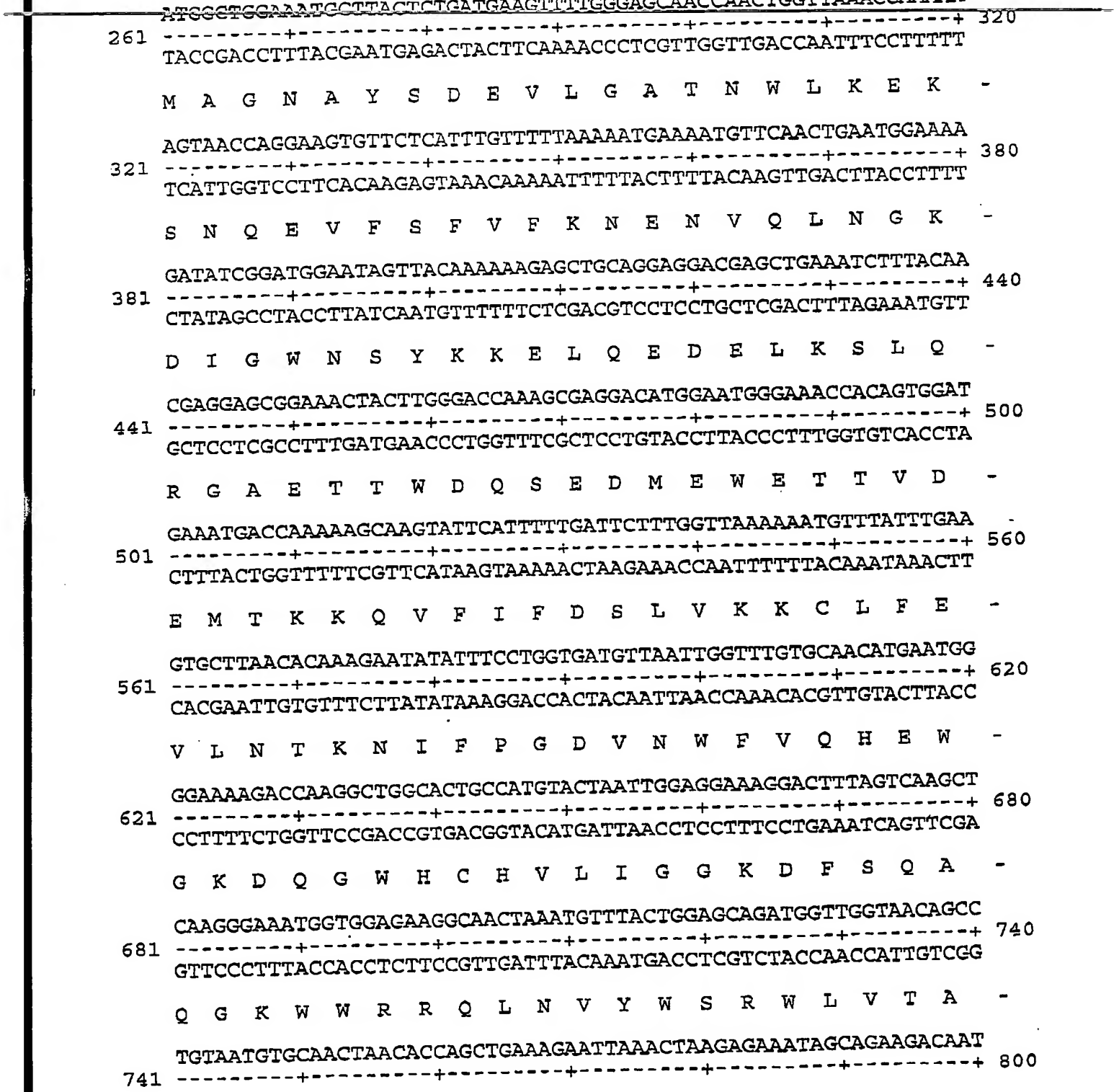


Fig. 1 (Fortsetzung 1)

```
      ACATTACACGTTGATTGTGGTCGACTTTCTTAATTTGATTCTCTTTATCGTCTTCTGTTA

      C  N  V  Q  L  T  P  A  E  R  I  K  L  R  E  I  A  E  D  N  -

      GAGTGGGTTACTCTACTTACTTATAAGCATAAGCAAACCAAAAAAGACTATACCAAGTGT
801   --------+--------+--------+--------+--------+--------+--------+ 860
      CTCACCCAATGAGATGAATGAATATTCGTATTCGTTTGGTTTTTTCTGATATGGTTCACA

      E  W  V  T  L  L  T  Y  K  H  K  Q  T  K  K  D  Y  T  K  C  -

      GTTCTTTTTGGAAACATGATTGCTTACTATTTTTTAACTAAAAAGAAAATAAGCACTAGT
861   --------+--------+--------+--------+--------+--------+--------+ 920
      CAAGAAAAACCTTTGTACTAACGAATGATAAAAAATTGATTTTTCTTTTATTCGTGATCA

      V  L  F  G  N  M  I  A  Y  Y  F  L  T  K  K  K  I  S  T  S  -

      CCACCAAGAGACGGAGGCTATTTTCTTAGCAGTGACTCTGGCTGGAAAACTAACTTTTTA
921   --------+--------+--------+--------+--------+--------+--------+ 980
      GGTGGTTCTCTGCCTCCGATAAAAGAATCGTCACTGAGACCGACCTTTTGATTGAAAAAT

      P  P  R  D  G  G  Y  F  L  S  S  D  S  G  W  K  T  N  F  L  -

      AAAGAAGGCGAGCGCCATCTAGTGAGCAAACTATACACTGATGACATGCGGCCAGAAACG
981   --------+--------+--------+--------+--------+--------+--------+ 1040
      TTTCTTCCGCTCGCGGTAGATCACTCGTTTGATATGTGACTACTGTACGCCGGTCTTTGC

      K  E  G  E  R  H  L  V  S  K  L  Y  T  D  D  M  R  P  E  T  -

      GTTGAAACCACAGTAACCACTGCGCAGGAAACTAAGCGCGGCAGAATTCAAACTAAAAAA
1041  --------+--------+--------+--------+--------+--------+--------+ 1100
      CAACTTTGGTGTCATTGGTGACGCGTCCTTTGATTCGCGCCGTCTTAAGTTTGATTTTTT

      V  E  T  T  V  T  T  A  Q  E  T  K  R  G  R  I  Q  T  K  K  -

      GAAGTTTCTATTAAAACTACACTTAAAGAGCTGGTGCATAAAAGAGTAACCTCACCAGAG
1101  --------+--------+--------+--------+--------+--------+--------+ 1160
      CTTCAAAGATAATTTTGATGTGAATTTCTCGACCACGTATTTTCTCATTGGAGTGGTCTC

      E  V  S  I  K  T  T  L  K  E  L  V  H  K  R  V  T  S  P  E  -

      GACTGGATGATGATGCAGCCAGACAGTTACATTGAAATGATGGCTCAACCAGGTGGAGAA
1161  --------+--------+--------+--------+--------+--------+--------+ 1220
      CTGACCTACTACTACGTCGGTCTGTCAATGTAACTTTACTACCGAGTTGGTCCACCTCTT

      D  W  M  M  M  Q  P  D  S  Y  I  E  M  M  A  Q  P  G  G  E  -

      AACCTGCTGAAAAATACGCTAGAGATTTGTACACTAACTCTAGCCAGAACCAAAACAGCA
1221  --------+--------+--------+--------+--------+--------+--------+ 1280
      TTGGACGACTTTTTATGCGATCTCTAAACATGTGATTGAGATCGGTCTTGGTTTTGTCGT

      N  L  L  K  N  T  L  E  I  C  T  L  T  L  A  R  T  K  T  A  -

      TTTGACTTAATTTTAGAAAAAGCTGAAACCAGCAAACTAACCAACTTTTCACTGCCTGAC
1281  --------+--------+--------+--------+--------+--------+--------+ 1340
      AAACTGAATTAAAATCTTTTTCGACTTTGGTCGTTTGATTGGTTGAAAAGTGACGGACTG

      F  D  L  I  L  E  K  A  E  T  S  K  L  T  N  F  S  L  P  D  -

      ACAAGAACCTGCAGAATTTTTGCTTTTCATGGCTGGAACTATGTTAAAGTTTGCCATGCT
1341  --------+--------+--------+--------+--------+--------+--------+ 1400
      TGTTCTTGGACGTCTTAAAAACGAAAAGTACCGACCTTGATACAATTTCAAACGGTACGA

      T  R  T  C  R  I  F  A  F  H  G  W  N  Y  V  K  V  C  H  A  -
```

Fig. 1 (Fortsetzung I

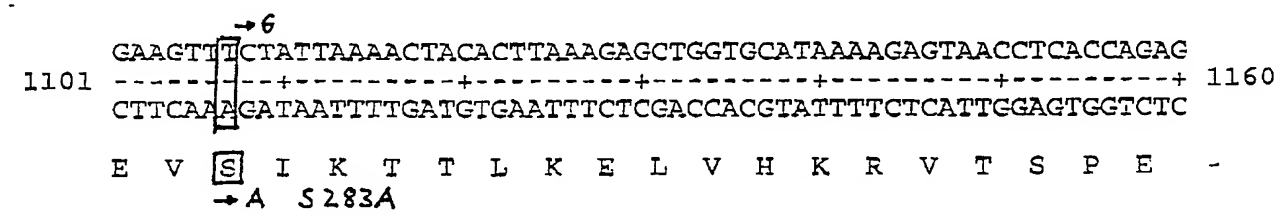
```
      ATTTGCTGTGTTTTAAACAGACAAGGAGGCAAAAGAAATACTGTTTTATTTCATGGACCA
1401  ---------+---------+---------+---------+---------+---------+ 1460
      TAAACGACACAAAATTTGTCTGTTCCTCCGTTTTCTTTATGACAAAATAAAGTACCTGGT

      I  C  C  V  L  N  R  Q  G  G  K  R  N  T  V  L  F  H  G  P  -

      GCCAGCACAGGCAAATCTATTATTGCACAAGCCATAGCACAAGCAGTTGGCAATGTTGGT
1461  ---------+---------+---------+---------+---------+---------+ 1520
      CGGTCGTGTCCGTTTAGATAATAACGTGTTCGGTATCGTGTTCGTCAACCGTTACAACCA

      A  S  T  G  K  S  I  I  A  Q  A  I  A  Q  A  V  G  N  V  G  -

      TGCTATAATGCAGCCAATGTAAACTTTCCATTTAATGACTGTACCAACAAGAACTTGATT
1521  ---------+---------+---------+---------+---------+---------+ 1580
      ACGATATTACGTCGGTTACATTTGAAAGGTAAATTACTGACATGGTTGTTCTTGAACTAA

      C  Y  N  A  A  N  V  N  F  P  F  N  D  C  T  N  K  N  L  I  -

      TGGGTAGAAGAAGCTGGTAACTTTGGACAGCAAGTAAACCAGTTTAAAGCCATTTGCTCT
1581  ---------+---------+---------+---------+---------+---------+ 1640
      ACCCATCTTCTTCGACCATTGAAACCTGTCGTTCATTTGGTCAAATTTCGGTAAACGAGA

      W  V  E  E  A  G  N  F  G  Q  Q  V  N  Q  F  K  A  I  C  S  -

      GGTCAAACTATTCGCATTGATCAAAAAGGAAAAGGCAGCAAACAGATTGAACCAACACCA
1641  ---------+---------+---------+---------+---------+---------+ 1700
      CCAGTTTGATAAGCGTAACTAGTTTTTCCTTTTCCGTCGTTTGTCTAACTTGGTTGTGGT

      G  Q  T  I  R  I  D  Q  K  G  K  G  S  K  Q  I  E  P  T  P  -

      GTCATCATGACCACAAATGAGAACATTACAGTGGTCAGAATAGGCTGCGAAGAAAGACCA
1701  ---------+---------+---------+---------+---------+---------+ 1760
      CAGTAGTACTGGTGTTTACTCTTGTAATGTCACCAGTCTTATCCGACGCTTCTTTCTGGT

      V  I  M  T  T  N  E  N  I  T  V  V  R  I  G  C  E  E  R  P  -

      GAACACACTCAACCAATCAGAGACAGAATGCTTAACATTCATCTAACACATACCTTGCCT
1761  ---------+---------+---------+---------+---------+---------+ 1820
      CTTGTGTGAGTTGGTTAGTCTCTGTCTTACGAATTGTAAGTAGATTGTGTATGGAACGGA

      E  H  T  Q  P  I  R  D  R  M  L  N  I  H  L  T  H  T  L  P  -

      GGTGACTTTGGTTTGGTTGACAAAAATGAATGGCCCATGATTTGTGCTTGGTTGGTAAAG
1821  ---------+---------+---------+---------+---------+---------+ 1880
      CCACTGAAACCAAACCAACTGTTTTTACTTACCGGGTACTAAACACGAACCAACCATTTC

      G  D  F  G  L  V  D  K  N  E  W  P  M  I  C  A  W  L  V  K  -

      AATGGTTACCAATCTACCATGGCAAGCTACTGTGCTAAATGGGGCAAAGTTCCTGATTGG
1881  ---------+---------+---------+---------+---------+---------+ 1940
      TTACCAATGGTTAGATGGTACCGTTCGATGACACGATTTACCCCGTTTCAAGGACTAACC

      N  G  Y  Q  S  T  M  A  S  Y  C  A  K  W  G  K  V  P  D  W  -

      TCAGAAAACTGGGCGGAGCCAAAGGTGCCAACTCCTATAAATTTACTAGGTTCGGCACGC
1941  ---------+---------+---------+---------+---------+---------+ 2000
      AGTCTTTTGACCCGCCTCGGTTTCCACGGTTGAGGATATTTAAATGATCCAAGCCGTGCG

      S  E  N  W  A  E  P  K  V  P  T  P  I  N  L  L  G  S  A  R  -

      TCACCATTCACGACACCGAAAAGTACGCCTCTCAGCCAGAACTATGCACTAACTCCACTT
2001  ---------+---------+---------+---------+---------+---------+ 2060
      AGTGGTAAGTGCTGTGGCTTTTCATGCGGAGAGTCGGTCTTGATACGTGATTGAGGTGAA
```

Fig. 1 (Fortsetzung II)

```
     S  P  F  T  T  P  K  S  T  P  L  S  Q  N  Y  A  L  T  P  L  -

     GCATCGGATCTCGAGGACCTGGCTTTAGAGCCTTGGAGCACACCAAATACTCCTGTTGCG
2061 ---------+---------+---------+---------+---------+---------+ 2120
     CGTAGCCTAGAGCTCCTGGACCGAAATCTCGGAACCTCGTGTGGTTTATGAGGACAACGC

     A  S  D  L  E  D  L  A  L  E  P  W  S  T  P  N  T  P  V  A  -

     GGCACTGCAGAAACCCAGAACACTGGGGAAGCTGGTTCCAAAGCCTGCCAAGATGGTCAA
2121 ---------+---------+---------+---------+---------+---------+ 2180
     CCGTGACGTCTTTGGGTCTTGTGACCCCTTCGACCAAGGTTTCGGACGGTTCTACCAGTT

     G  T  A  E  T  Q  N  T  G  E  A  G  S  K  A  C  Q  D  G  Q  -

     CTGAGCCCAACTTGGTCAGAGATCGAGGAGGATTTGAGAGCGTGCTTCGGTGCGGAACCG
2181 ---------+---------+---------+---------+---------+---------+ 2240
     GACTCGGGTTGAACCAGTCTCTAGCTCCTCCTAAACTCTCGCACGAAGCCACGCCTTGGC

     L  S  P  T  W  S  E  I  E  E  D  L  R  A  C  F  G  A  E  P  -

     TTGAAGAAAGACTTCAGCGAGCCGCTGAACTTGGACTAA
2241 ---------+---------+---------+--------- 2279
     AACTTCTTTCTGAAGTCGCTCGGCGACTTGAACCTGATT

     L  K  K  D  F  S  E  P  L  N  L  D  *  -
```

Fig. 1.1

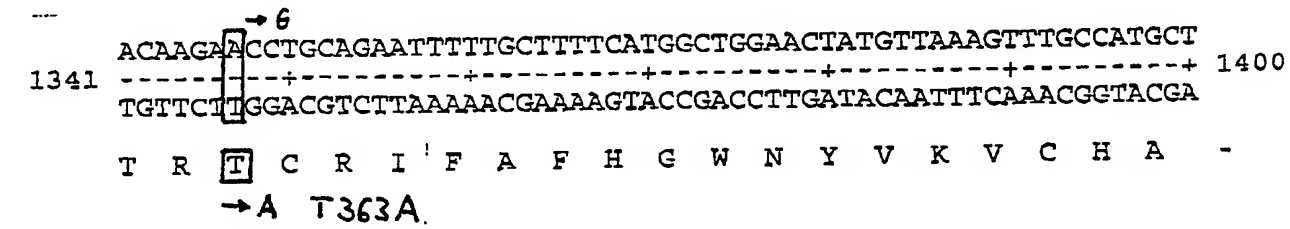
1100 - 261 Wildtype-NS1-Sequence




1161 - 2279 Wildtype-NS1-Sequence

Fig. 1.2

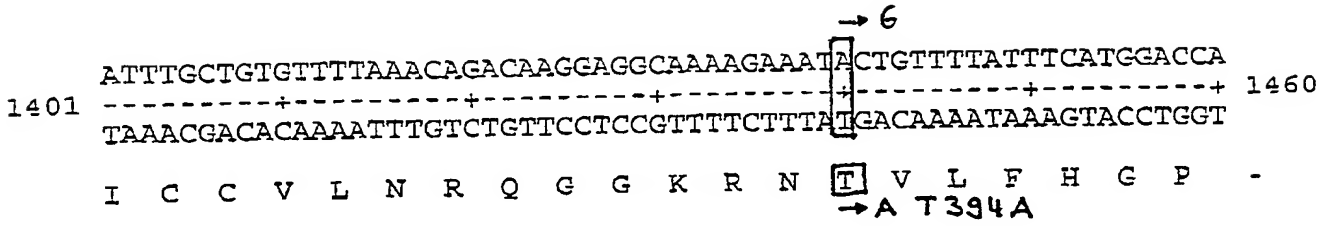
1340 - 261 Wildtype-NS1-Sequence




1401 - 2279 Wildtype-NS1-Sequence

Fig. 1.3

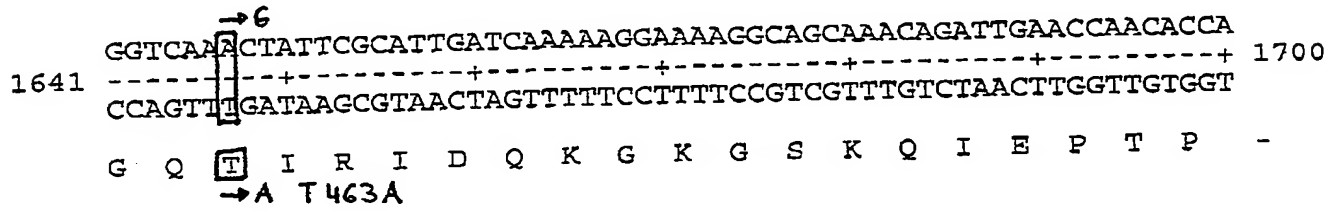
1400 - 261 Wildtype-NS1-Sequence




1461 - 2279 Wildtype-NS1-Sequence

Fig. 1.4

1640 - 261 Wildtype-NS1-Sequence




1701 - 2279 Wildtype-NS1-Sequence

## Summary

The present invention relates to a parvovirus NS1 variant having a shifted equilibrium between the DNA replication and transcription activities (a) and the cytotoxicity activity (b). Furthermore, this invention relates to DNAs coding for these parvovirus NS1 variants and methods of producing them. Additionally, this invention concerns antibodies directed against the parvovirus NS1 variants as well as the use of the DNAs and the parvovirus NS1 variants.